PROCEEDINGS OF
THE PHYSIOLOGICAL SOCIETY

OXFORD MEETING

24–25 September 1982

DEMONSTRATIONS

Lightnesses, colours and the reaction of wavelength selective cells in monkey striate cortex

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This was a demonstration of some of Land's retinex experiments (Land, 1974) undertaken solely to assist in understanding the experimental conditions used to study cells in monkey striate cortex (see communication by Zeki at this meeting).

A multicoloured display was illuminated with all three projectors, each equipped with a band-pass filter, one passing long-wave (red) light only, the other middle-wave (green) light only and the third short-wave (blue) light only. When the display is illuminated with long-wave light only, the entire display acquires a red wash and some areas will appear very light whereas others very dark. The red, white, magenta and yellow areas will all appear light and human observers cannot predict which of these will be red when the display is later illuminated with all three projectors, *nor will the appearance of red depend upon the sequence with which the display is alternately illuminated with long-wave light and with light of all three wavebands.* The same is true for illumination with short- and middle-wave light only, except that the lightnesses will change, e.g. a red area will appear very dark when illuminated with middle-wave light only, whereas it appeared very light when illuminated with long-wave light only. In general, full colours appear only when the display is illuminated with light of all three wavebands. It was also shown that the identical effects are obtained if narrow-band interference filters (Ditric Co. Inc.) are substituted for the band-pass filters.

Split-screen video television recordings, in colour, of the responses of single-wavelength selective cells in monkey striate cortex to these conditions of illumination were demonstrated simultaneously.

This work was supported by the Science and Engineering Research Council.

REFERENCE

LAND, E. H. (1974). *Proc. Roy. Inst. Gt. Britain* **47**, 23.

A simple electrical analogue of CO_2 transport in the body

By I. R. Summers and Jane Ward*. *Department of Physics, Exeter University and *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

Computer-assisted models of the respiratory system have been described previously (Grodins, Buell & Bart, 1967; Saunders, Bali & Carson, 1980) and certain aspects of arterial P_{CO_2} have been modelled graphically (Cunningham, 1975).

We have devised a simple electrical analogue which allows visualization of both the immediate and delayed effects on mixed venous and arterial P_{CO_2} of changes in one or more of the variables known to affect CO_2 transport. The variables included in the model are tidal volume, respiratory frequency, tissue CO_2 production, cardiac output and inspired P_{CO_2}. The model involves no computation and its output is thus an accessible and instantaneous 'thinking aid' when considering the mean level or the oscillating component of arterial P_{CO_2} as possible control signals.

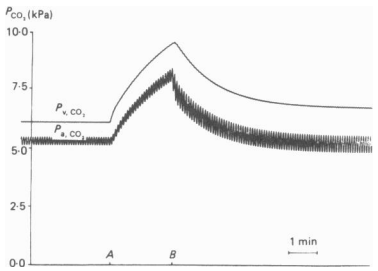

Fig. 1. Time course of changes in modelled mixed venous and arterial P_{CO_2} following doubling of tissue CO_2 production at *A*, and doubling of tidal volume at *B*.

The tissues are represented by a single compartment which receives arterial blood from the lungs after a delay. The tissues add CO_2 to the blood which returns, after a further delay, as mixed venous blood to the lung. The lungs, which are represented as a single alveolar compartment, intermittently discharge part of their contents to atmosphere.

This simple model is represented by an electrical circuit and Fig. 1 is an example of its output. At *A*, CO_2 production is doubled, all other variables remaining unchanged. Both mixed venous and arterial P_{CO_2} rise and the oscillations of P_{a,CO_2} about the mean level increase in amplitude and slope. At *B* tidal volume is doubled. Mean level of arterial P_{CO_2} returns to its original level while mixed venous P_{CO_2} falls but remains higher than its original level. The oscillations of P_{a,CO_2} continue to increase finally reaching twice their original size.

REFERENCES

Cunningham, D. J. C. (1975). *Nature, Lond.* **253**, 440–442.
Grodins, F. S., Buell, J. & Bart, A. J. (1967). *J. appl. Physiol.* **22**, 260–276.
Saunders, K. B., Bali, H. N. & Carson, E. R. (1980). *J. theor. Biol.* **84**, 135–161.

Self-sealing silicone rubber membranes for long-term implants

By G. S. Brindley. *MRC Neurological Prostheses Unit, Institute of Psychiatry, De Crespigny Park, London SE5 8AF*

Implants that must remain in the body for many years may contain liquids that need to be withdrawn or added to after implantation. This can be done by a hollow needle through the skin if the implant's surface includes a self-sealing membrane (e.g. Rohde, Blackshear, Varco & Buchwald, 1975). A self-sealing membrane that can easily be made from very durable materials is shown in Fig. 1. Its essential feature is the alternation of silicone rubbers of low and high compliance. It withstands 120 cmH_2O without leaking after being punctured one hundred times with a 21 gauge (0·76 mm) needle.

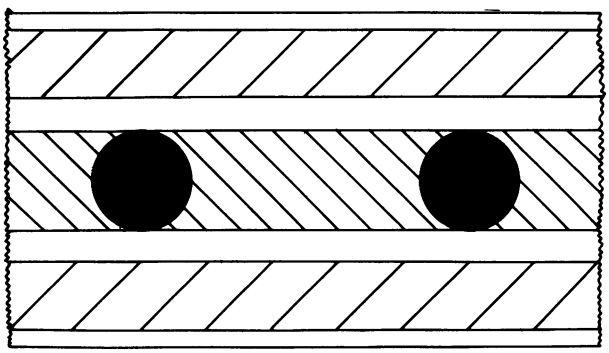

Fig. 1. Section through a two-way self-sealing membrane. Solid black: stainless steel reinforcing wires. Dexter hatched: Dow Corning 891 (stiff). White: Silastic sheet (stiff). Sinister hatched: Dow Corning 734 (soft). Total thickness 2·5 mm. If self-sealing is required only for pressure from below, the top two layers can be omitted.

REFERENCE

Rohde, T. D ., Blackshear, P. J., Varco, R. L. & Buchwald, H. (1975). *Trans. Amer. Artif. Int. Organs*, **21**, 510–514.

A low frequency variable symmetry waveform generator

By W. J. Bannister. *University Laboratory of Physiology, Parks Road, Oxford OX1 3PT*

In the course of research into the response of the human auditory system to frequency and amplitude modulations, the ability to change the parameters of the modulating waveform quickly and easily became increasingly necessary.

A programmable generator was designed to produce triangles, square waves and trapezoids whose half periods are independently variable over a range of 1 msec to 10 sec. The rise and fall time can be selected over the same range, thus giving complete control over the slope, periodicity and symmetry.

The output waveform can be passed through a sine-shaper to produce sinewaves, or trapezoids with sinusoidal rise and fall.

The generator can be triggered and stopped by 5/OV.TTL pulses, or gated by means of a 5 V level, and the number of cycles output per triggered sequence can be preset from 1 to 99.

When averaging evoked responses, it is necessary to synchronize the acquisition to the modulation waveform, and so a 5-V pulse is available at the start of each cycle.

The output is variable up to 10 V P/P at 600 Ω.

A peak amplitude detector for audio frequencies

By W. J. Bannister. *University Laboratory of Physiology, Parks Road, Oxford OX1 3PT*

This device was designed initially to extract information about amplitude modulations present in the complex and irregular waveforms in speech.

The positive going zero crossing of each cycle of the carrier waveform is used as a trigger, and the following positive peak value is detected and 'held' until the next peak arrives and updates the display. The input range is between 25 mV and 2·5 V at carrier frequencies up to 10 kHz.

A L.E.D. indicator is lit when the device is being triggered, and when the input is too small to initiate a reading, the output is switched to zero after 25 msec.

The output is linearly proportional to the positive peak value of the input, within the range stated, and is useful for calibrating AM waveforms.

A computer-linked direct-reading osteometric board

By A. F. Rogers and Juliet M. Rogers. *Department of Physiology, University Walk, Bristol BS8 1TD and Department of Medicine, The Royal Infirmary, Maudlin Street, Bristol*

During paleopathological investigations of skeletal material large numbers of measurements such as length and diameters of bones may have to be made (Rogers,

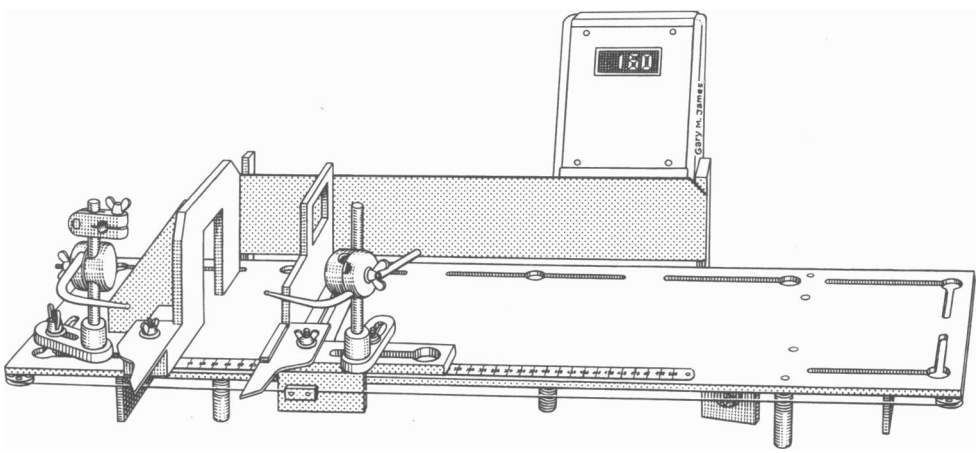

Fig. 1. Osteometric board.

Watt & Dieppe, 1981). With the apparatus demonstrated, measurements are made by the displacement of a saddle along a base plate and sensed by the rotation of a potentiometer. The saddle moving on ball races is kept precisely at right angles by wire cables attached at front and rear which, after passing round pulleys at each end of the table, pass diagonally under the table to the opposite corner and along the other side, thus ensuring precisely true movement. Underneath the table one cable passes round a drum on the spindle of a precision potentiometer across which 1000 mV is applied. The output of the potentiometer may then be adjusted to read displacement in mm as mV. The output on the slider of the potentiometer, which indicates exactly the displacement of the saddle, is read by a digital voltmeter (Analogic, AN 2574) connected in the ratiometric mode and itself linked to the computer, which interrogates the voltmeter and records millivoltage as a length in millimetres.

We are grateful to P. Harper for programming advice and to the Sir Halley Stewart Research Trust for financial support.

REFERENCE

ROGERS, J., WATT, I. & DIEPPE, P. (1981). *Br. med. J.* **283**, 19–26.

Simple device for labelling negatives during photomicrography

BY G. C. VAN DEN BOS, R. H. BUITENWEG, J. G. MEIJER and D. VERBEEK. *Department of Physiology, The Free University of Amsterdam, 1081 BT Amsterdam, The Netherlands*

When large numbers of photographs are taken of rather similar microscopic sections identification of negatives can be difficult. Electronic data backs (Minolta, Revue) which allow letters and/or numbers to be put on the film during exposure

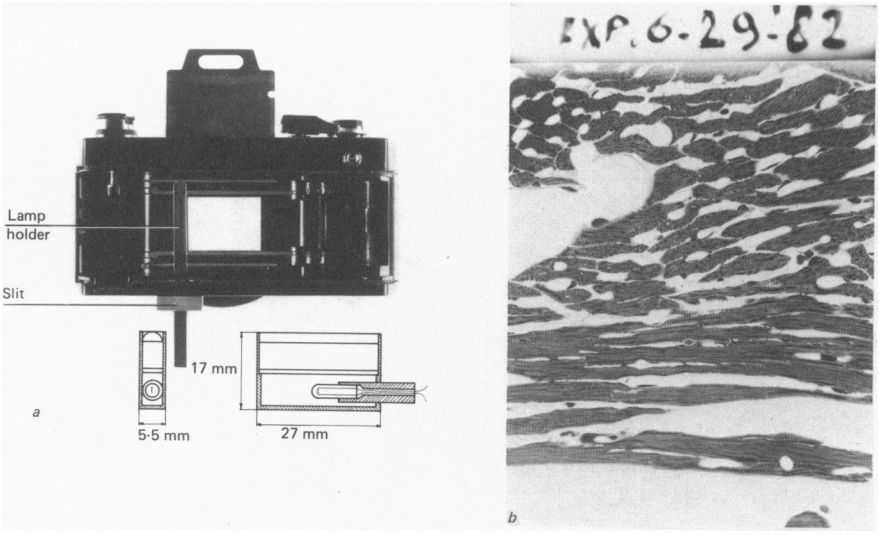

Fig. 1

are expensive and do not fit conventional microscope cameras. Leitz has developed a device for their MDa camera through which a strip of plastic can be slid into the camera back. The data on this strip is photographed on one side of the negative together with the object. In our hands this system is only effective if the photographed object does not extend into the area where the strip is projected.

In our Zeiss M35 microscope camera we have made a similar system (Fig. 1*a* top) modified in the following way. Under the slit for the data strip we have glued to the inside of the camera body a small black rectangular container (Fig. 1*a* bottom). Its open end is covered by a small Plexiglass cylinder lens. The light from the miniature lightbulb inside the container projects the data of the strip on the overlying film (Fig. 1*b*). The camera shutter control also triggers a circuit switching the lamp on and off for correct exposure. The strips can be made of any strong transparent plastic. The end extending from the camera body has to be blackened otherwise outside light is conducted via the plastic onto the film.

REFERENCE

ERNST LEITZ, GMBH – D 6330 Wetzlar. Einlageblatt zur Anleitung 110-72b.

An apparatus for automatically measuring tissue weight changes induced by alterations in the bathing medium

BY C. CLAIRE AICKIN, ALISON F. BRADING and R. MANCHANDA. *University Department of Pharmacology, South Parks Road, Oxford OX1 3QT*

The tissue is mounted on a stainless-steel holder, rigidly suspended from a sensitive transducer with an output to a pen recorder. The tissue is immersed in a small, jacketed organ bath through which the bathing fluid is circulated by pumping through a

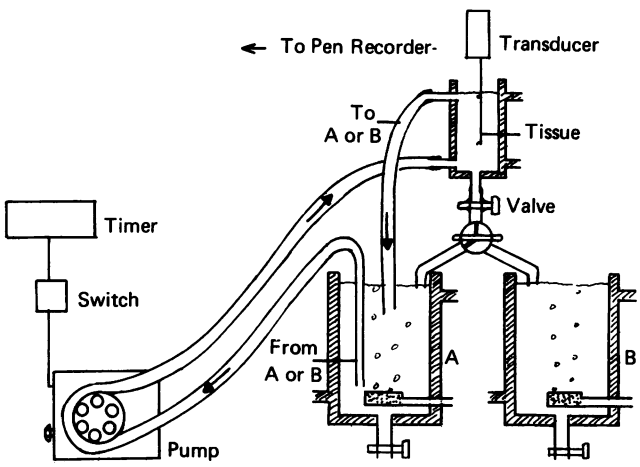

Fig. 1. Diagram of apparatus. Temperature control is achieved by circulating heated water through the hatched area.

high-output pump (≏ 200 ml./min) made in the laboratory. Fluid is pumped from a heated reservoir in which it is equilibrated with the desired gas mixture, into the bottom of the organ bath from whence it returns simultaneously via an overflow and a valved outlet at the bottom of the bath. The pump rate is adjustable and it can be switched off automatically for variable durations and at the desired frequency set by a timer. When the pump is switched off, the bath drains at a rate determined by the valve, leaving the tissue suspended in air for long enough for the pen recorder to give a stable reading of the weight of the tissue and holder. Changes of solution are achieved by switching the input and output tubes to a second reservoir. Changes in recorded weight reflect changes in the tissue weight since the amount of adhering fluid remains relatively constant because the bath drains at a constant rate, and the tissue is mounted isometrically. The actual amount of fluid adhering can be determined by using a radioactive extracellular marker.

A new method of mounting a strip of smooth muscle for investigation with intracellular micro-electrodes

By C. Claire Aickin and P. Flaxman. *University Department of Pharmacology, Oxford OX1 3QT*

In vitro muscle preparations are conventionally secured by pins. Although a simple and effective method, it has two drawbacks. First, no adjustment can b

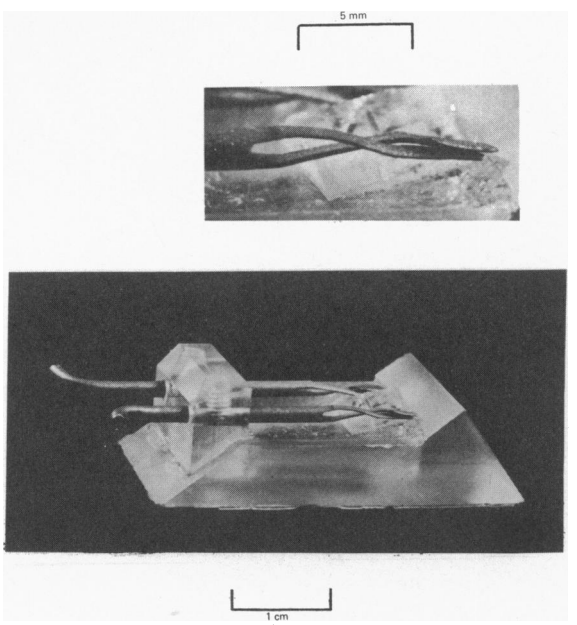

Fig. 1. Photographs of the rotating clamp showing detail of the jaws at the top and the complete arrangement for mounting a strip of smooth muscle at the bottom. The clamps are secured with nylon screws to prevent easy rotation. The Perspex slide on which they are mounted is shaped so that the muscle can be viewed horizontally in the experimental chamber.

without increasing the damage to the preparation and second, only a limited amount of tension can be applied before the fixing pins cut through the preparation like cheese wire. The new mounting system overcomes both disadvantages and has increased the success rate for long-term (in excess of 2 hr) continuous intracellular recording in smooth muscle cells.

The basis of the method is a rotating clamp shown in Fig. 1, made from 17 gauge stainless steel needle tubing. The jaws of the clamp can be held apart with forceps while the strip of muscle (ideally 1–1·5 cm long, 1–2 mm wide) is slipped between them. The closed jaws compress the muscle sufficiently for maximal tension to be maintained without cutting through the muscle. A pair of these clamps is mounted on a Perspex slide either side of a silicone-rubber wedge (see Fig. 1) over which the muscle is stretched. Thus initial adjustments are made out of the restricted area of the experimental chamber and final adjustment is made by rotation of either clamp after the slide has been transferred to the chamber.

Demonstration of oscillatory variations in $[Ca]_i$ and membrane currents in a computer model of Ca-induced Ca release in mammalian Purkinje fibre and ventricular muscle

By Dario DiFrancesco*, George Hart† and Denis Noble†. **Istituto di Fisiologia Generale, Milano, Italy and †University Laboratory of Physiology, Oxford OX1 3PT*

We have previously calculated (DiFrancesco, Hart & Noble, 1982) that a secondary release of calcium following repolarization in the model of DiFrancesco & Noble (1981, 1982) would generate the transient inward current (TI) observed experimentally in Purkinje fibres (Lederer & Tsien, 1976). We have now modelled the release process itself by assuming that an internal store requires two Ca^{2+} ions to bind to each site to release Ca^{2+}. This release store is reprimed by an uptake store, presumed to be the sarcoplasmic reticulum. The parameters of the model were adjusted to give $[Ca]_i$ transients similar to those recorded experimentally during the action potential with aequorin (Allen & Kurihara, 1980; Weir & Isenberg, 1982). If the background level of $[Ca]_i$ is raised, this model then produces regenerative and oscillatory release, as inferred from experiments in skinned fibres by Fabiato & Fabiato (1975). In the intact cell model, the simplest way of achieving this condition is to raise $[Na]_i$ and indeed, when the activity of the Na-K pump is reduced and membrane currents are computed following step depolarizations, we obtain damped oscillatory currents, the frequency and magnitude of which closely resemble those observed experimentally in pump-blocked preparations. In this model, the main oscillatory current is that of the electrogenic Na–Ca exchange process, but this does not exclude the involvement of other Ca-activated currents since, as inferred by Kass, Lederer, Tsien & Weingart (1978), the essential oscillatory process involves the intracellular stores and does not greatly depend on surface membrane currents.

Our conclusion is that the known and likely properties of the intracellular storage and release mechanisms are sufficient to explain slow oscillatory Ca changes and membrane currents responsible for after-contractions and extrasystoles.

The demonstration showed these and related results on a PDP 11/34 computer.

We acknowledge the support of the M.R.C. and British Heart Foundation.

REFERENCES

ALLEN, D. G. & KURIHARA, S. (1980). *Europ. Heart J.* **1**, 5–15.
DIFRANCESCO, D., HART, G. & NOBLE, D. (1982). *J. Physiol.* **328**, 15–16*P*.
DIFRANCESCO, D. & NOBLE, D. (1981). *J. Physiol.* **320**, 25–26*P*.
DIFRANCESCO, D. & NOBLE, D. (1982). In *Cardiac Rate & Rhythm*, ed. BOUMAN, L. N. & JONGSMA, H. J., pp. 93–128. The Hague: Martinus Nijhoff.
FABIATO, A. & FABIATO, F. (1975). *J. Physiol.* **249**, 469–495.
KASS, R. S., LEDERER, W. J., TSIEN, R. W. & WEINGART, R. (1978). *J. Physiol.* **281**, 187–208.
LEDERER, W. J. & TSIEN, R. W. (1976). *J. Physiol.* **263**, 73–100.
WEIR, W. G. & ISENBERG, G. (1982). *Pflügers Arch.* **392**, 284–290.

A low-cost microcomputer system for spike-processing

BY J. LEENDERTZ and D. M. WRIGHT. *Department of Anatomy, The Medical School, University of Bristol, Bristol BS8 1TD*

The equipment demonstrated utilizes a microcomputer as an alternative to a conventional hardwired ratemeter for the quantification of neuronal activity. The system is based on an Acorn Atom microcomputer which was chosen because of its low cost and the suitability of its hardware and software for simple interfacing with external electronic equipment.

In our prototype the number of action potentials occurring in successive epochs are counted and the value displayed on a video monitor together with a cumulative count and elapsed time. The Acorn Atom has two eight-bit input/output ports, one of which is used as a bi-directional data bus and the other to switch on individual components in sequence. The addition of a small number of external components (e.g. counter chips, D/A chips and latches) allows the output of data in an analogue form (as a frequency/time histogram) which can be displayed on a chart recorder. Variables, such as epoch time and D/A sensitivity, are read in from switches via latches hung onto the bus and can therefore be altered without interference with the running of the program. The program is stored on a C-MOS memory chip, which can be maintained almost indefinitely by means of a small on-board battery so that the program is instantly available at switch-on. This avoids the inconvenience of cassette loading and the expense of disk storage.

The cost of this system compares favourably with the cost of a hard-wired system and can perform many additional functions. The whole program may be written in BASIC making it fairly straightforward for the system to be altered or reconfigured by someone without detailed electronic knowledge. With greater programming knowledge BASIC and assembler code can be mixed within the program enabling critical sections, where speed or space is important, to be written in assembler while for simplicity and clarity the remainder is written in BASIC. In this way we are developing further programs to provide additional functions such as interspike interval analysis, pulse generation, timing and control of external equipment. These will then be stored in the C-MOS memory chip and located on separate plug-in boards.

This work was supported by A.R.C.

An inexpensive, precise computer-controlled bicycle ergometer for racing cyclists

By E. G. Merrill. *University Laboratory of Physiology, Oxford OX1 3PT*

This ergometer was developed in order to measure more precisely the work capacity of racing cyclists. Existing instruments either cannot be adjusted to the exact geometry of the racing cyclist's own machine (and therefore cannot measure the specific fitness developed during hundreds of hours of training on a particular bicycle), or require frequent calibration.

The present ergometer was constructed from a racing bicycle frame, with adjustable saddle height and adjustable handlebar stem length and height. The bike is mounted on a stand which allows limited lateral movement. A reproducible resistance is produced by a commercially available squirrel-cage fan, which is driven by the bicycle's rear wheel. The resistance increases rapidly with speed (as is the case for the cyclist working against air resistance), approximating actual racing loads. A large electric fan is used to cool the cyclist.

Cast-iron weights have been added to the rim of the rear wheel to add mechanical inertia, allowing a more natural pedalling action. Rear-wheel revolutions are sensed by a proximity detector.

A simple, inexpensive microprocessor system (Sinclair ZX 81) was deliberately chosen to keep the cost of the total system low. Programs have been written in machine code which allow, via a CRT display for the racer, various race situations to be simulated. The computer calculates actual work performed, from the instantaneous rate of rear wheel revolution, and compares this to the pattern of demand contained in the program. Plots of error *vs.* time, cumulative and average power, etc. are produced at the completion of the test.

Some fourteen of these ergometers are now in use by coaches in the British Cycling Coaching Scheme in a national physiological assessment program for racing cyclists. Two are in regular use in studies of the energy costs of racing for competitive cyclists.

A suite of programmes for analysing spontaneous cell discharges

By A. Angel. *Department of Physiology, The University, Sheffield S10 2TN*

Time-shared micro-spectrophotometric measurements using arsenazo III in large crustacean muscle fibres

By C. C. Ashley and M. V. Thomas. *University Laboratory of Physiology, Parks Road, Oxford OX1 3PT*

Single striated muscle fibres from the barnacle *Balanus nubilus* were cannulated and axially microinjected with a solution of the metallochromic indicator arsenazo III (ASIII) in 100 mM-K-(*N*-tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid) (K-TES) buffer (pH 7·3), and left over 1–2 hr for radial diffusion. An axially inserted electrode was employed (Ashley, Franciolini, Lea & Lignon 1979; Ashley & Lignon, 1981) and the preparation was stimulated by voltage-clamp techniques. The fibre was aligned upon a 'bridge' of Sylgard resin 184 (Dow Corning) through which

the incident light was channelled. The time-sharing spectrophotometer design is described elsewhere (Thomas, 1982) apart from the following minor modifications. The rotor was designed to carry six interference filters (13 mm d., *ca.* 10 nm band width – Ditric Optics, U.S.A.) mounted in 16 mm d. brass sleeves, the weights of which were adjusted so that it was balanced irrespective of the filters employed. This allowed ready substitution of filters for either ASIII or other metallochromic indicators. The rotor speed was *ca.* 200 rev/sec so that individual wavelengths were sampled at 5-msec intervals.

The light collection was achieved by a fused bundle of optical fibres (Image Conduit – Edmund Scientific, U.S.A., distributed by Astrophil, Dublin) of 3·5 mm overall d. The end adjacent to the fibre was heated to give a blade-shaped collecting area (4 mm × 0·5 mm). A photodiode (EG & G, type PV100) was mounted at the other end of the light guide. The remainder of the electronic circuitry was as described previously (Thomas, 1982). Movement artefacts upon stimulation of the fibre were reduced by stretching the fibre tautly over the 'Sylgard bridge', and were further minimized by careful positioning of the collecting light guide while observing the absorbance change either at the λ570 nm isosbestic point or differentially at the λ720–λ750 nm wavelength pair. Absorbance signals attributed mainly to Ca^{2+} were detected differentially at either λ660–λ690 nm or λ660–λ720 nm. In some experiments fibres were injected with ASIII and aequorin. In the latter case, a conventional photomultiplier assembly was also employed, and in these experiments a comparison could be made of the two methods for measuring free Ca^{2+} in the same muscle cell.

Work supported by the Medical Research Council.

REFERENCES

ASHLEY, C. C., FRANCIOLINI, F., LEA, T. J. & LIGNON, J. (1979). *J. Physiol.* **296**, 71*P*.
ASHLEY, C. C. & LIGNON, J. (1981). *J. Physiol.* **318**, 10–11*P*.
THOMAS, M. V. (1982). *Techniques in Calcium Research*. New York: Academic Press (in the Press).

An undergraduate class using blood gas electrodes to determine dissociation curves

BY D. J. C. CUNNINGHAM, D. F. O'CONNOR and E. S. PETERSEN. *University Laboratory of Physiology, Oxford OX1 3PT*

The mammalian muscle spindle (abridged edition videotape)

BY I. A. BOYD. *Institute of Physiology, University of Glasgow, Glasgow G12 8QQ*

A superfusion apparatus to study field stimulation of smooth muscle from mammalian urinary bladder

BY ALISON F. BRADING and G. N. A. SIBLEY. *Department of Pharmacology, University of Oxford, OX1 3QT and Department of Urology, Churchill Hospital, Oxford*

Brading & Sneddon (1980) described a superfusion apparatus for studying contractile responses of guinea-pig *Taenia coli*. This has been modified and improved to

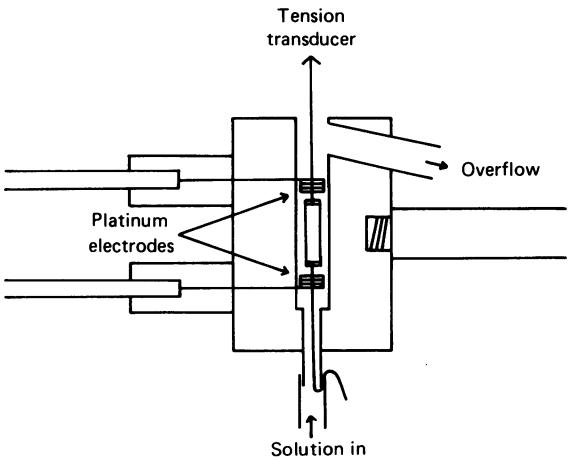

Fig. 1. Diagram of organ bath used for field stimulation in the superfusion apparatus.

enable responses to field stimulation to be studied in strips taken from mammalian urinary bladder.

Oxygenated solutions are pumped by a multi-channel peristaltic pump through an oil-filled heating bath to the tissues at a flow rate of 1 ml./min. The tissue, measuring approximately 7 mm × 1·5 mm unstretched, is suspended by silk threads between two platinum ring electrodes 1 cm apart in a cylindrical organ bath constructed from Perspex (see Fig. 1), and with a capacity of 0·2 ml. Isometric tension changes are measured using Pioden UF1 tension transducers, and after amplification the contractions are recorded on a Watanabe multi-channel pen recorder. Using six organ baths, six tissues can be studied simultaneously.

The electrical impulses for field stimulation are delivered from a Grass S48 stimulator. A simple switch-box enables the output of the stimulator to be connected to each of the organ chambers in turn. Tetrodotoxin is used in these experiments on field stimulation to distinguish between nerve-mediated contractile responses and those due to direct muscle stimulation.

The superfusion apparatus allows rapid solution changes and accurate, short exposure times to drugs. It is therefore possible to compare the responses to field stimulation and to different drug applications in the same tissues, and to study the effects of continuously superfused agonists and antagonists on the field stimulation response.

The support of the M.R.C. is gratefully acknowledged.

REFERENCE

BRADING, A. F. & SNEDDON, P. (1980) *Br. J. Pharmac.* **70**, 229–240.

Analysis of cerebellar neurone spike trains recorded in monkeys performing a visual tracking task

By R. I. Kitney, R. C. Miall, P. Riddell and J. F. Stein. *University Laboratory of Physiology, Oxford OX1 3PT and Department of Electrical Engineering, Imperial College, London*

We have been recording from neurones in the cerebellum of monkeys trained to track a visual target with a hand-held lever, in an attempt to deduce what operations the cerebellum may perform on visual input in order to control motor output. However even when the discharge of these neurones is clearly related to target or monkey movement there are formidable problems of interpretation. Raw records of spike frequency contain much 'noise'; but conventional techniques employed to increase signal-to-noise ratios, such as averaging or Fourier methods, lose much important information because they cannot accommodate features of the records which change with time. Cerebellar operations seem to be both non-linear and non-stationary, and discharges of movement-related cells may cease to relate to target or monkey movements altogether for brief periods. Hence it is important not to lose the original time structure, whatever technique for noise reduction is employed, if we wish to understand what these neurones may be doing.

We have therefore made use of two techniques commonly employed in signal analysis to try to overcome these problems. The first consists of simply filtering the spike frequency records at the fundamental frequency of the target movement, or ten times that frequency. This has shown that the firing of neurones in both lateral and intermediate cerebellum is related to position, velocity, acceleration and higher-order components of monkey movement, but that these relationships change with time.

Autoregressive spectral analysis allows us to examine the frequency components of monkey movement and neuronal discharges over short time periods, e.g. less than 1 movement cycle. We have found that these frequency components may change over the course of a few cycles. Successive frequency spectra were calculated every second. Perspective plots of these enabled us to identify easily changes in frequency components with time. The results suggest that 'entrainment' interactions between target movement and natural frequency components of the monkey's motor system may underlie some of the frequency shifts which we observe.

Ionic exchange in isolated mammalian ventricular cells measured with extracellular ion-selective electrodes

By C. H. Fry and J. P. T. Ward. *Department of Physiology, St. Thomas' Hospital, London SE1 7EH*

Measurement of ionic exchange in isolated cells with ion-selective electrodes can offer several experimental advantages. Diffusion spaces are removed, *net* fluxes of several ions can be simultaneously measured and the dynamics of exchange accurately evaluated. We illustrated such advantages by demonstrating one aspect of ionic exchange in isolated ventricular cells.

Experiments were performed at 37 °C in a water-jacketted chamber, the base of

which is an oxygen electrode (Fry & Williams, 1979). Cells were prepared according to Powell, Terrar & Twist (1980). A sample of cells was resuspended in a HEPES-buffered medium, containing 140 mM-K^+, 10 mM-glucose, a calcium ion buffer and variable Na^+ and Mg^{2+}, and was then introduced into the chamber. Ion-selective electrodes inserted in a stopper could then monitor the ionic activity of the suspension medium. A decrease of activity was interpreted as an uptake by the cells.

Addition of digitonin (final concentration 20 μM) resulted in a prompt increase in oxygen consumption by the preparation; a net uptake of calcium was measured if the suspension calcium activity, a_{Ca}, was greater than 1 μM. The magnitude and initial rate of the uptake increased as suspension a_{Ca} was raised to about 100 μM, in addition they were sensitive to Mg^{2+} ions and less sensitive to Na^+ ions. Addition of ruthenium red (final concentration 10 μM) revealed an efflux of calcium under these conditions superimposed upon the net uptake. This efflux was sensitive to Na^+ ions over the range 4–30 mM yielding a Hill coefficient of 3.

A number of criteria suggest that the observed calcium exchange is across the mitochondrial membranes. These include the absolute requirement of oxygen, its augmentation by respiratory substrates, the effect of respiratory uncouplers and the stoichiometric counter-movement of protons. The absence of any effect of methyl-xanthines suggests a negligible contribution from the sarcoplasmic reticulum.

One advantage of using such a method to elucidate cellular ionic metabolism is that fluxes can be related to cell number so that the cellular capacity of any system can be calculated. In addition these intact cells allow pretreatment of the preparation before measurements are made.

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REFERENCES

Fry, C. H. & Williams, A. J. (1979). *J. Physiol.* **289**, 2–3*P*.
Powell, T., Terrar, D. & Twist, V. W. (1980). *J. Physiol.* **302**, 131–153.

Use of an inexpensive microcomputer for evaluating examples from a simple kind of control theory

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12-bit analogue-to-digital conversion and subsequent current density analysis of extracellular potentials recorded from the hippocampus using a microcomputer

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Programs have been written in Z80 assembly language and FORTRAN to run under CP/M on a Vector Graphic microcomputer. The first program performs 12-bit analogue-to-digital conversion using a 25 KHz, S-100 bus compatible board from California Data Corporation.

Evoked extracellular profiles from different depths into the CA1 area of the hippocampus are digitized and averaged on-line and stored on a disk. The 500-point profile is displayed on a video monitor to allow measurement or expansion of any part of the waveform. For each averaged waveform, data from up to five user-specified points along the profile can be accumulated and stored separately. This package is a useful extension to the one demonstrated by Hornsey & Wheal (1981).

A second program accepts data from analogue plots of extracellular field profiles using the digitizing facility of a Hewlett-Packard 7225A plotter. Data may also be entered from a disk file or the keyboard. This program executes 7-point smoothing of the data and computation of the second derivative using a least-squares method described by Savitzky & Golay (1964) modified by Steinier, Termonia & Deltour (1972). The second derivative approximates the current density (Nicholson & Freeman, 1975). At each stage the data may be replotted or stored on a disk.

REFERENCES

HORNSEY, D. G. & WHEAL, H. V. (1981). *J. Physiol.* **315**, 5–6*P*.
NICHOLSON, C. & FREEMAN, J. A. (1975). *J. Neurophysiol.* **38**, 356–368.
SAVITZKY, A. & GOLAY, M. J. E. (1964). *Analyt. Chem.* **36**, 1627–1639.
STEINIER, J., TERMONIA, Y. & DELTOUR, J. (1972). *Analyt. Chem.* **44**, 1906–1909.

COMMUNICATIONS

HRP intracellular recordings from single frog optic axons: preliminary observations on the functional morphology of ganglion cells, their axons and central arbors

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At present there is only circumstantial evidence that different types of visual response recorded in the frog tectum are generated by ganglion cells with various morphologies, and that the tectal arbors of each terminate at different depths in the tectum (Chung, Bliss & Keating, 1974; Maturana, Lettvin, McCulloch & Pitts, 1960).

Fig. 1. Dimming unit axonal arbor.